Remarks

Claim amendments

Claim 16 is amended in response to an objection for dependence from a previously cancelled claim (claim 13). Support for the amendment is found in at least original claims 13 and 16. Claim 25 is amended in response to a definiteness rejection. Support for this amendment is found in at least original claims 21, 24, and 25. Because these amendments addresses objections expressly set forth in the Office Action and place the application in better form for consideration upon appeal, their entry after a Final Rejection is proper. See MPEP 714.12 and 37 C.F.R. § 1.116(b)(1).

These amendments are made without disclaimer of subject matter and solely to advance prosecution. No new matter is added by these amendments.

Enablement

The claims have been rejected for allegedly lacking enablement commensurate with their full scope. The Examiner has agreed that the claims are enabled for use with fibroblasts as the primary cell used for nuclear transfer, but has disputed whether they are enabled for other cell types.

At the outset, the rejection of claims 5 and 10 is improper because those claims recite that the primary cell used for nuclear transfer is a fibroblast. Because it is agreed that the claims are enabled for use with the recited cell type, the rejections as stated are clearly inapplicable against these claims.

As to the remaining claims, Applicants respectfully traverse and request reconsideration and withdrawal based on the following.

Cells other than fibroblasts have sufficient proliferative potential to successfully undergo genetic modification prior to senescence.

Claims 7, 14, 21-25, 27-36, and 106 were rejected as allegedly non-enabled for encompassing genetic modification of cultured mammalian primary cells other than fibroblasts. The Examiner contends that other than fibroblasts, every primary cell type has insufficient proliferative potential (prior to senescence) to be cultured long enough for successful genetic modification. The sole evidence for this contention is the Denning publication[1]. Denning disclose a method of culturing and genetically modifying primary cells that is estimated to require cells proliferate for 45 population doublings. Denning also reports that cultures of disaggregated whole pig and sheep embryos gave initially mixed cultures, but after about 12 population doublings the cultures were predominantly fibroblasts, and further reports that primary pig cell cultures derived from disaggregated gut, kidney, lung, and mesonephros yielded cell populations that become senescent within 40 population doublings. The Examiner has taken the position that because some cell types may be unable to proliferate sufficiently to complete the Denning transformation protocol, the claims are not enabled.

Contrary to the alleged basis of rejection, the art teaches numerous mortal cell types (in addition to fibroblasts) that are able to be cultured and proliferate beyond the estimated 45 generations used in the Denning protocol. For example, primary RPE cells can proliferate for 58 population doublings before senescence[2], satellite cells can proliferate for up to 65 population doublings before senescence[3], and human large vessel derived endothelial cells can proliferate for 65-75 population doublings before senescence[4]. Thus, numerous cell types are able to proliferate for the 45 population doublings used in the Denning protocol. Therefore, the rejection lacks factual basis because it is predicated on the faulty assumption that only fibroblasts have sufficient proliferative potential for use in the Denning protocol.

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[1] Cloning and Stem Cells. 2001;3(4):221-31.

[2] Matsunaga *et al.*, Invest Ophthalmol Vis Sci. 1999 Jan;40(1):197-202, pg., 198, left column.

[3] van der Ven, "Skeletal Muscle," in Koller *et al.*, eds., *Primary Mesenchymal Cells*, Kluwer Academic Publishers, 2001, pg. 73 (citing Decary, Hum Gene Ther. 1997 Aug 10;8(12):1429-38).

[4] Williams, "Microvascular endothelium from adipose tissue," in Bicknell, ed., *Endothelial Cell Culture*, Cambridge University Press, 1996, pgs. 97-99.

Additionally, rejection is improper for only considering one possible method of genetically modifying cells. The genetic modifications recited in the claims encompass numerous other methods including those that require substantially fewer population doublings. For example, the Denning protocol could be readily modified to reduce the number of population doublings required by omitting steps which are unnecessary for obtaining a transformant (such as cryopreservation and recovery, which is performed twice during the Denning protocol). Denning cites Clark[5] to further explain how 45 population doublings are consumed in their protocol. Clark states that just 15 doublings are sufficient to give enough cells for transformation using this method, and that even fewer doublings are required when using a more efficient DNA delivery method such as microinjection (pg. 268, right column). The art teaches that microinjection can yield transformants with efficiency as high as 31% [6], thus just a few population doublings can give ample numbers of cells for transformation by microinjection. Further, it is well known in the art to use alternatives to antibiotic selection, such as GFP expression, to identify transformants, thereby avoiding using population doublings for selection and clonal expansion of transformants. Other DNA delivery methods, such as use of viral vectors, can also achieve greater transformation efficiency than the Denning methods. Thus, numerous methods are available in the art which use far fewer population doublings than the Denning method. Indeed, the art teaches successful genetic modification of numerous mortal cell types, including cultured RPE cells[7]; muscle, liver, brain, and retina *in vivo*[8]; and cultured satellite cells[9]. Because the art teaches methods for genetic modification of numerous mortal cell types, the claims are enabled for genetic modification of other cell types than fibroblasts.

As discussed in Applicant's Remarks dated December 7, 2009, enablement does not require every imaginable combination of elements to result in an operative method. Rather, the test is "**whether a skilled person could determine which embodiments that were conceived, but not yet made, would be inoperative or operative with expenditure of no more effort than is normally required in the art**." MPEP 8th ed., rev. 6, § 2164.08(b) (*citing Atlas Powder Co. v. E.I. du Pont de Nemours & Co.*, 750 F.2d 1569, 1577, 224 USPQ 409, 414 (Fed. Cir.

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[5] Transgenic Res. 2000;9(4-5):263-75, of record.
[6] Folger, Mol Cell Biol. 1982 Nov;2(11):1372-87, Table 1.
[7] Bodnar *et al.*, Science. 1998 Jan 16;279(5349):349-52.
[8] Xiao *et al.*,, J Virol. 1999 May;73(5):3994-4003.

1984)) (emphasis added). It is undisputed that those of skill in the art can readily determine how many population doublings a cell can undergo prior to senescence (*e.g.*, Matsunaga, *supra*). It is also undisputed that those of skill in the art can readily determine the number of population doublings that may be consumed by a given protocol for genetic modification (*e.g.*, Denning, Clark, *supra*). Thus, those of skill in the art may readily determine which methods of genetic modification to use for a given type of cells. Moreover, the art provides numerous working examples of genetic modification of mortal cell types other than fibroblasts (*e.g.*, Bodnar, Xiao, and Cudré-Mauroux, *supra*). Therefore, the present claims are enabled for genetic modification of cell types other than fibroblasts, and accordingly, reconsideration and withdrawal of the rejection is respectfully solicited.

The cited evidence fails to demonstrate inoperability of telomere restoration and increasing the number of remaining population doublings using cell types other than fibroblasts

Claims 1, 3-8, 10-12, 14-16, 21-25, 27-36, and 106 (*i.e.*, all pending claims) have been rejected as allegedly not enabled for encompassing nuclear transfer using nuclear donor cells other than fibroblasts. As noted above, the rejection of claims 5 and 10 is improper because those claims recite that the primary cell used for nuclear transfer is a fibroblast, which the Examiner has stated is enabled.

As to the remaining claims, it is undisputed that the physical steps involved in the claimed could readily be performed irrespective of cell type. However, the Examiner has rejected the claims as non-enabled based on alleged uncertainty as to whether telomeres would be restored or remaining population doublings would be increased when using a nuclear donor cell other than a fibroblast. This rejection is based on the shortened telomeres of "Dolly," which was cloned from quiescent cultured adult mammary cells, reported by Shiels *et al.*[10] Though Shiels *et al.* was the uncontradicted teaching in the art at the time of filing, it stands in disagreement with the working and prophetic[11] examples in the present disclosure and a series of

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[9] Cudré-Mauroux *et al.*., Hum Gene Ther. 2003 Nov 1;14(16):1525-33.

[10] Though not cited in the Office Action, the statements regarding Dolly's telomeres are understood to refer to Shiels *et al.*, "Analysis of telomere length in Dolly, a sheep derived by nuclear transfer," Cloning 1999;1(2):119-25.

[11] *See, e.g.*, para. 0048 of the specification as published (PGPub. No. 20040180430).

post-filing publications in which multiple research labs demonstrate telomere restoration in cloned animals derived from multiple cell types, including fibroblasts (Tian[12], Betts[13], Jiang[14], Jeon[15]), cumulus cells (Tian, Wakayama[16]), and granulosa cells (Betts). Thus, the references show that the telomere shortening reported in Shiels *et al.* must simply be regarded as an aberration. Indeed, even one of the clones reported in Shiels *et al.* (clone 6LL7) had telomeres statistically indistinguishable from age-matched controls. *See* pg. 123, left column ("6LL7 is not significantly different at 95% confidence limits from age-matched controls ($p < 0.088$), despite having a smaller mean TRF size"). Indeed, regarding Dolly's shortened telomeres, Tian further states that:

> **the shortened telomeres found in Dolly are likely to be an exception**, whereas the restored telomere pattern found in cattle and mice may be the general result for cloned animals.

Tian et al., pg. 273, middle column (emphasis added). Thus, when Shiels *et al.* is taken together with these post-filing publications, the very worst that one could reasonably infer is that cloning almost always restores telomeres but might occasionally fail. However, the mere existence of occasional failures does not justify an enablement rejection.

Moreover, Dolly was derived by a different method than the method of the present claims. Even if Dolly's shortened telomeres were viewed as something more than just an aberrant result, Dolly was derived using **quiescent** donor cells. In contrast, the present claims recite **senescent** or near-senescent donor cells. Thus the short telomeres of Dolly do not demonstrate inoperability of the presently claimed method because that clone was created by a different method.

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[12] Tian et al., "Normal telomere lengths found in cloned cattle," Nature Genetics, vol. 26, Nov. 2000, pages 272-273.

[13] Betts et al., "Reprogramming of telomerase activity and rebuilding of telomere length in cloned cattle," Proc Natl Acad Sci Usa, vol. 98, 2001, pages 1077-1082.

[14] Jiang L et al., "Telomere Lengths in Cloned Transgenic Pigs," Biology Of Reproduction, vol. 70, 2004, 1589–1593.

[15] Jeon HY et al., "The analysis of telomere length and telomerase activity in cloned pigs and cows," Mol Reprod Dev, vol. 71, 2005, pages 315-320.

[16] Wakayama et al., "Cloning of mice to six generations," Nature. 2000 Sep 21;407(6802):318-319.

The Examiner further cites the discussion in the post-filing publication, Lanza *et al.* Science 288:665-9 (2000), which shows elongation of telomeres in animals cloned from senescent cells. Lanza discusses several possible explanations the apparent contrast to the Shiels *et al.* results. Though one of the possible explanations mentioned was differences in cell types (which was cited by the Examiner as alleged evidence in support of the rejection), several other possible reasons for the differences were also given, including that the animals were cloned by different methods (use of quiescent versus senescent cells). However, the mere mention of several possibilities does not support the Examiner's contention that a particular one of them must be true. Rather, the aforementioned post-filing teachings in the art by Tian, Betts, Jiang, Jeon and Wakayama have shown that the short telomeres of Dolly are the exception rather than the rule.

Definiteness

Claim 25 has been rejected as allegedly indefinite for recitation of "a primary mammalian cell that has been genetically altered," specifically for not explicitly stating a baseline for determining the alteration. Applicants believe that those of skill in the art would readily understand the meaning of the term "genetically altered" including the baseline for determining the alteration. Nonetheless, the claims are amended to recite "providing a genetically altered primary mammalian cell, wherein said ~~that has been~~ genetically altered primary cell is genetically altered relative to a primary cell from which it was derived" which explicitly provides a baseline, rendering the rejection moot. Moreover, as to the statement that the genetic alteration encompasses multiple possibilities, Applicants respectfully disagree that merely encompassing multiple possibilities is a proper basis an indefiniteness rejection (see Applicant's remarks dated Dec. 17, 2009, pages 17-18). Accordingly, reconsideration and withdrawal of the rejection is respectfully solicited.

Double-patenting

Applicants continue to request that the rejection be held in abeyance pending the Examiner's indication that the claims are otherwise in condition for allowance.

CONCLUSION

In view of the foregoing, all claims are believed to be in condition for allowance. In the event that any additional issues remain, or if it would expedite the prosecution of this application, the Examiner is respectfully invited to contact the undersigned (direct line, 703-714-7645).

The Director is hereby authorized to charge any fees (including fees for extensions of time that may be required for consideration of this paper or to maintain the pendency of this application), or credit any overpayments, to our **Deposit Account No. 50-0206.**

Respectfully submitted,
HUNTON & WILLIAMS LLP

Date: 8/24/10

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APPENDICES

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